

Since this date I have seen a small *Calotes* seize and eat a small conspicuous orange-red insect, apparently a "lady-bird."

The behaviour of these reptiles certainly does not appear to afford support to the belief that the *butterflies*, at any rate, usually considered nauseous, are distasteful to them.

A Note on the Nature of the Substance formed during fermentation, from which Indigo Blue is eventually formed in Indigo Manufacture; and on Indigo Brown.—By SURG.-LT.-COL. G. S. A. RANKING, B.A., M.D., M.R.A.S.

[Read March, 1896.]

Indigo liquor when properly fermented is a greenish yellow infusion having a very marked greenish fluorescence. In reaction it varies, though the reaction is faintly alkaline when the liquor is most favourably fermented. A distinctly acid reaction always indicates unfavourable fermentation, and results in loss of produce of Indigo-blue.

It contains a substance in solution which forms a yellow solution with alkalis, and from this yellow solution Indigo-blue may be very readily obtained by simple agitation with air.

Now the nature of this Indigo-forming substance has been hitherto undecided. That it very closely resembles Indigo-white cannot be denied, as will be seen from a comparison of the reactions of Vat-liquor and solutions of Indigo-white respectively with metallic salts, herein-after set out in tabular form. (*See Table page 51.*)

But there are certain difficulties in the way of any theory which would declare them to be identically the same, of which one is this, that Indigo-white is well known to be insoluble except in alkalis, whereas it is certain that in acid Vat-liquor the Indigo-forming body is present in solution.

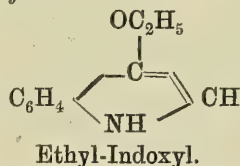
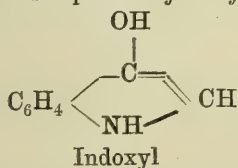
For many years I held the opinion that the substance present in the Vat-liquor after fermentation is so nearly allied to Indigo-white as to be practically identical with that body, and I considered that it might be an isomer of Indigo-white, which differed from that body by being soluble in acids, as well as in alkalis; I have, however, as a result of further research, come to the conclusion that the body present differs from Indigo-white in composition, though in its reactions with metallic salts it is apparently identical, and I have been led to conclude that it is probably Indoxyl (C_8H_7NO) a body containing one more atom of Hydrogen than does Indigo-white.

There are only two substances known to chemists which yield Indigo-blue on agitation of their solutions with air. These are:—

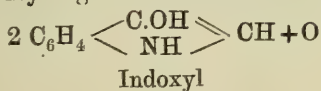
(a) Indigo-white (C_8H_6NO) soluble in alkalis to yellow solution, playing the part of a weak acid.

(b) Indoxyl (C_8H_7NO) soluble in acids or alkalis, and its solution in hot water showing a yellowish green fluorescence.

“It is simultaneously an acid and a base: its alkaline solution absorbs Oxygen from the air with formation of Indigo-blue: which is also formed when ferric chloride is added to its hydrochloric acid solution.”* Now it requires very little further enquiry, before we are struck with the similarity of the physical characters of this body and those of the body existing in Indigo Vat-liquor after fermentation. When we look a little further, and enquire into the chemical behaviour of Indoxyl, we find that it contains two atoms of replaceable Hydrogen. It forms derivatives in which one of these atoms is displaced, thus Ethyl-Indoxyl C_8H_6NO (C_2H_5) is Indoxyl in which one atom of Hydrogen is replaced by Ethyl: graphically—



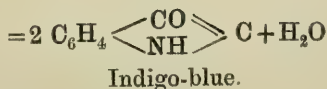
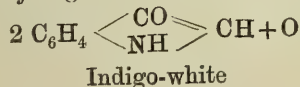
From this we may infer that a Potass-Indoxyl (C_8H_6NOK) Sod-Indoxyl ($C_8H_6NO Na$). Ammon Indoxyl ($C_8H_6NO Am$) are theoretically possible. We may also notice that if we simply remove this atom of Hydrogen we should convert Indoxyl into Indigo-white, thus



Indigo-white.



and this indicates that the above would represent the graphic formula of Indigo-white. Further oxidation would give us Indigo-blue, the atom of Hydrogen contained in the CH group being removed: thus †



* Roscoe and Schorlemmer, Vol. III. p. 40.

† R. and S., Vol. III. page 42.

Now in fermented Indigo-liquor we have a body present which forms by the action of air Indigo-blue. It must therefore be, so far as is known at present, one of two bodies: Indigo-white or Indoxyl. Which of these two it is the following considerations will help to decide.

It is soluble in water and the solution has generally a faintly alkaline reaction but may be acid. It therefore seems not to be Indigo-white.

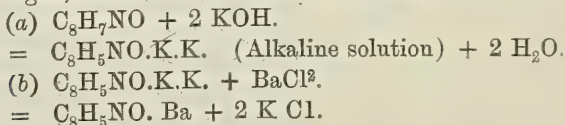
On the other hand its solution shews a very characteristic green fluorescence, and, moreover, if treated with HCl and Fe_2Cl_6 yields Indigo-blue, which fact strongly supports the view that Indoxyl is present: the above reaction being one of the characteristics of Indoxyl (*vide R. and S. loc. cit.*)

But supposing it to be Indoxyl, as it really seems that it may be, how can we account for the fact that it reacts with the salts of the metals just as does Indigo-white. To explain this we must briefly reconsider our position. We found that Indoxyl has two replaceable Hydrogen atoms, one in the OH group, the other in the CH group, and that if both these atoms are removed Indigo-blue is left.

First consider what occurs when an alkaline solution of Indigo-white is treated with a solution of a metallic salt. (See Table Column I).

A double compound is formed consisting of the metal and Indigo-blue, the metal having secondarily replaced the replaceable atom of Hydrogen. These compounds may be shewn to consist of Indigo-blue and the metallic base, by treatment with strong sulphuric acid* and subsequent addition of water, when Indigo-blue will be thrown down and the metal remain in combination with the acid.

Now I imagine that, in the case of the Vat-liquor containing Indoxyl, what happens is this, we recollect it has two atoms of replaceable Hydrogen, and both of these are removed, thus



The result being ultimately the same as in the case of white Indigo, with this difference that the Indoxyl is the more powerful reducing agent of the two, and needs more oxygen; so that the ultimate reactions of the metals with alkaline solutions of Indoxyl and Indigo-

* 1. $\text{C}_8\text{H}_5\text{NO. Ba} + \text{H}_2\text{SO}_4 + \text{O} = \text{C}_8\text{H}_5\text{NO} + \text{Ba SO}_4 + \text{H}_2\text{O}$, atmospheric oxidation occurring as part of the reaction.

N. B.—It should be noticed that the composition of the double compound of Indigo-blue and metal in the case of Barium points to an original compound in which there were two atoms of replaceable Hydrogen, such as Indoxyl.

white are the same, the only difference being in the proportions of the reagents required: * and this would explain how it is that the Indigo Vat-liquor while giving the reactions of Indigo-white with alkalis and metals, still may have an acid reaction originally, if the body present is Indoxyl $C_8H_7NO=$ or ψ Indoxyl. Which we might call an acid Indigo-white on the analogy of the acid carbonates.

Having regard to the fact that CO_2 is largely present during Indigo fermentation it is not improbable that at some stage Indoxylic acid



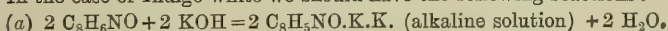
is present, but as this, by the splitting off of CO_2 , yields Indoxyl, and in alkaline solution behaves just as does Indoxyl, yielding Indigo-blue by oxidation, this does not in any way affect the foregoing explanation of the reactions occurring during the manufacturing process.

The following table shews the reactions above referred to.

Table shewing comparison of reactions of Indigo Vat-liquor after due fermentation, with those of Reduced or white Indigo.

The reactions of Reduced Indigo <i>vide</i> Watts Dicty. of Chemistry, ed. 1865, Vol. III.	Reactions of fermented Indigo liquor with the reagents mentioned.	
	Reagent.	Result.
Earth metals and heavy metals give a white ppt. turning blue in air.	Ba Cl ₂	White ppt. becoming dark blue in air.
Magnesium compound is partly soluble, partly white ppt. turning blue, partly a yellow solution.	Mg SO ₄	White ppt. becoming blue in air. Filtrate yellow.
Aluminium compound white, but rapidly turns blue in the filter.	Al ₂ (SO ₄) ₃	White ppt. becoming blue rapidly.
Manganous compound dirty green, yields no sublimate of Indigo-blue.	Mn Cl ₂	Bluish green ppt. No sublimate on heating.
Zinc compound is white, rapidly blue in air and then yields a sublimate of Indigo-blue when heated.	Zn SO ₄	White ppt. becoming rapidly blue. <i>No sublimate obtained.</i>
Lead compound white and slightly crystalline, turns rapidly blue on exposure to air, if then heated it detonates slightly and yields reduced lead.	Pb(NO ₃) ₂	White ppt. <i>inclining to green</i> , blue on exposure to air, on heating deflagrates yielding Pb.
Ferrous compound is white, quickly blue on exposure to air: if then heated, yields no sublimate of Indigo-blue.	Fe SO ₄	Whitish green ppt., passing through green to blue (also some ferric hydrate ppt. owing to impurity of ferrous salt.)

* In the case of Indigo-white we should have the following reactions :



(b) As in case of Indoxyl-Barium compound.

The reactions of Reduced Indigo <i>vide</i> Watts Dicty. of Chemistry, ed. 1865, Vol. III.	Reactions of fermented Indigo liquor with the reagents mentioned.	
	Reagent.	Result.
Cobalt compound grass green yields no sublimate of Indigo-blue.	Co Cl ₂	Grass green ppt. becoming dark bluish green, yields no subli- mate of Indigo-blue.
Nitrate of silver produces in aqueous so- lution of the potassium compound a ppt. which is at first transparent brown and then becomes black; it is not acted upon by the air, but when heated produces a gentle explosion and yields a sublimate of Indigo-blue, and a resi- due of metallic silver.	Ag NO ₃	Brown ppt. becoming black glossy looking not altered in air: when heated after drying detonated slightly* yielding sublimate of Indigo-blue. Residue metallic silver or oxide of silver: not sol. in NH ⁴ HO— Sol. in HNO ³ repptd. by HCl ∴ Ag.

N.B.—All the above reactions were obtained in Vat-liquor rendered distinctly alkaline with KOH dilute, with which a yellow solution was formed rapidly oxidising with formation of Indigo-blue.

It is therefore necessary to add the alkali to each portion of the Vat-liquor separately to get trustworthy results. The similarity of the reactions of fermented Indigo-liquor and of a solution of reduced Indigo is seen in the above series. The italics in the third column shew the only points of difference observed.

A few more remarks upon the above precipitates obtained from fermented Indigo Vat-liquor will be of interest. All the precipitates enumerated in the above Table, with one exception, when dried at 100°C and treated in a porcelain capsule with strong H₂SO₄ yield a dark green solution, which upon the addition of water deposits Indigo-blue, the filtrate in the case of the soluble sulphates giving the characteristic reactions of the metallic base used as a precipitant.

There is, however, one noticeable exception to this rule in the case of the Sodium-silver-indigo compound, which does not detonate on heating and yields a sublimate of a *brown colour, not of Indigo-blue*—on treatment with concentrated H₂SO₄ it turns brown and yields no Indigo-blue on the addition of water. I have thought it interesting to examine the composition of this salt, and have arrived at the conclusion that the Indigo in it is not Indigo-blue, but one of the other compounds,

* Absence of $\begin{matrix} \text{COOH} \\ \text{COOH} \end{matrix}$ proved.

probably one of the so-called "Indigo-browns" of which as I shall shew there appear to be at least two.

This Sodium-silver-indigo compound is like the potassium-silver-indigo compound in appearance, but is browner, and does not detonate when heated, nor does it yield a sublimate of Indigo-blue. It is insoluble in water, alcohol, or ether, partially soluble in dilute nitric acid with decomposition of the acid. Partially soluble in NH_4HO dilute, with which it forms a dark brown solution leaving a black residue in powder; less easily soluble in Na OH or KOH to brown solution. Sodium chloride gives no precipitate. The ammoniacal solution is brown by reflected light, deep vinous red by transmitted light, it passes through filter paper unchanged (A), leaving a fine black powder on the filter (B).

(1) A. Filtrate of NH_4HO solution. Added HCl in excess, no ppt. With HNO_3 dil. decolorized on boiling, and gives with HCl a white ppt. sol. in NH_4HO ; HNO_3 driven off by evaporating to dryness, confirmed with K_2CrO_4 .

(2) Portion of filtrate evaporated to dryness over a water bath was greenish black with metallic lustre, when heated gave a faint brown sublimate not yielding Indigo-blue on treatment with H_2SO_4 conc. and subsequent addition of water. This lustrous residue dissolved in HNO_3 dil. on boiling, and gave the reactions of silver with HCl and with K_2CrO_4 .

(3) Portion dried in a crucible and ignited gave a dark violet residue with metallic lustre.

Washed with strong H_2SO_4 the violet portions turned green (trace of Indigo); then washed well with H_2O and then digested the residue in NH_4HO , but nothing was dissolved. \therefore no Ag_2O .

Again washed with H_2O and dissolved in HNO_3 the solution became turbid on adding HCl dil. and cleared up on adding AmO \therefore Ag , confirmed by K_2CrO_4 .

It thus appears that the portion of the ppt. of the Sodium-silver-indigo compound which is soluble in Ammonia, contains traces of Indigo-blue but the majority of the Indigo is present as one of the Indigo-browns to be hereinafter referred to, decolorizing with HNO_3 with characteristic smell of hyperoxidized Indigo products.

B. The portion of the Sodium-silver-indigo ppt. which does not dissolve in NH_4HO is left on the filter in the form of a fine black powder. This portion was thoroughly washed with weak Ammonia solution and finally with H_2O and dried at 100°C . When dry it formed a very fine grey powder, so fine as not to be capable of being scraped off the filter. Filter digested in boiling HNO_3 dil. 10% the grey ppt. dissolves to a colourless solution giving the reactions of Ag . No residue left. The ppt. insoluble in NH_4HO is thus proved to be metallic silver.

It is thus evident that in the case of the Sodium-silver-indigo salt the resulting compound is made up of metallic silver, traces of Ag_2O and an Indigo-brown insoluble in water and alcohol but soluble in NH_3 *

It would appear therefore that while with the other metals the Indoxyl combines with them and is oxidised to Indigo-blue, in the case of the Sodium-silver-indigo compound the Indigo is further oxidised, reducing the silver to the metallic condition, itself being a brown substance soluble in NH_4HO but not soluble in H_2O . Now this is interesting as throwing light upon the way in which Indigo-brown (a source of great loss in manufacture) is formed. The Indigo-brown however which results from the improper fermentation of Indigo liquor is soluble in water.

Now it occurred to me to try whether, by a process of reduction from this last product, we could get a less highly oxidised Indigo-brown, and the results are most interesting. The solid extract obtained from dry Indigo leaves a brown resinous looking substance soluble in water, obtained by evaporation to dryness of a watery extract of dry Indigo leaves, was reduced in acid solution by means of Zinc: a brown powder was thrown down and on separation by filtration and washing was found to be insoluble in water but soluble in NH_4HO thus behaving exactly like the brown Indigo compound found to be in the Sodium-indigo-silver compound.

The inference is therefore possibly just that this Indigo-brown occupies a position intermediate between Indigo-blue and the soluble Indigo-brown resulting from the action of heat, alkalis, or improper fermentation on Indigo Vat-liquor: inasmuch as it is produced by a degree of oxidation which reduces the silver to the metallic state; and is also obtainable by reducing agencies from the soluble Indigo-brown formed in dry Indigo leaves.

It is therefore a further possible deduction from the facts observed, that an improper oxidation of the Indigo Vat-liquor will result in Indigo-brown instead of Indigo-blue: possibly this occurs during manufacture owing to the prolonged "beating" which is necessary to secure the deposition of the Indigo-blue in cases where fermentation has not gone on properly, as it is only formed in a small quantity in laboratory experiments with the plants, where all the stages can be more carefully controlled than is possible in actual practice owing to the various distances from which plant has to be brought and the irregular steeping which of necessity results.

However this may be I am certain that alkalinity † of the water

* Cf. Indihumin (Schunck.)

† Acidity of the steeping water leads to the production of a red compound soluble in water from which it may be separated by agitation with Ether ?Indirubin.

used to macerate the Indigo plant, the application of heat during maceration ("steeping"), and last but not least prolonged "beating" in order to oxidise imperfectly fermented Vat-liquor, all result in the formation of the soluble Indigo-brown and should therefore be avoided in manufacture.

The gases evolved during fermentation I have found to be principally Carbon dioxide and Nitrogen in the following proportions $\text{CO}_2 = 31$ per cent. $\text{N} = 62$ per cent. Hydrogen forms a very small proportion of the evolved gases, only 6.75 per cent.

Marsh gas also seems to be very sparingly generated during fermentation of Indigo. I propose to extend my observations on the evolved gases at some future opportunity.